1/7
FIG. 1

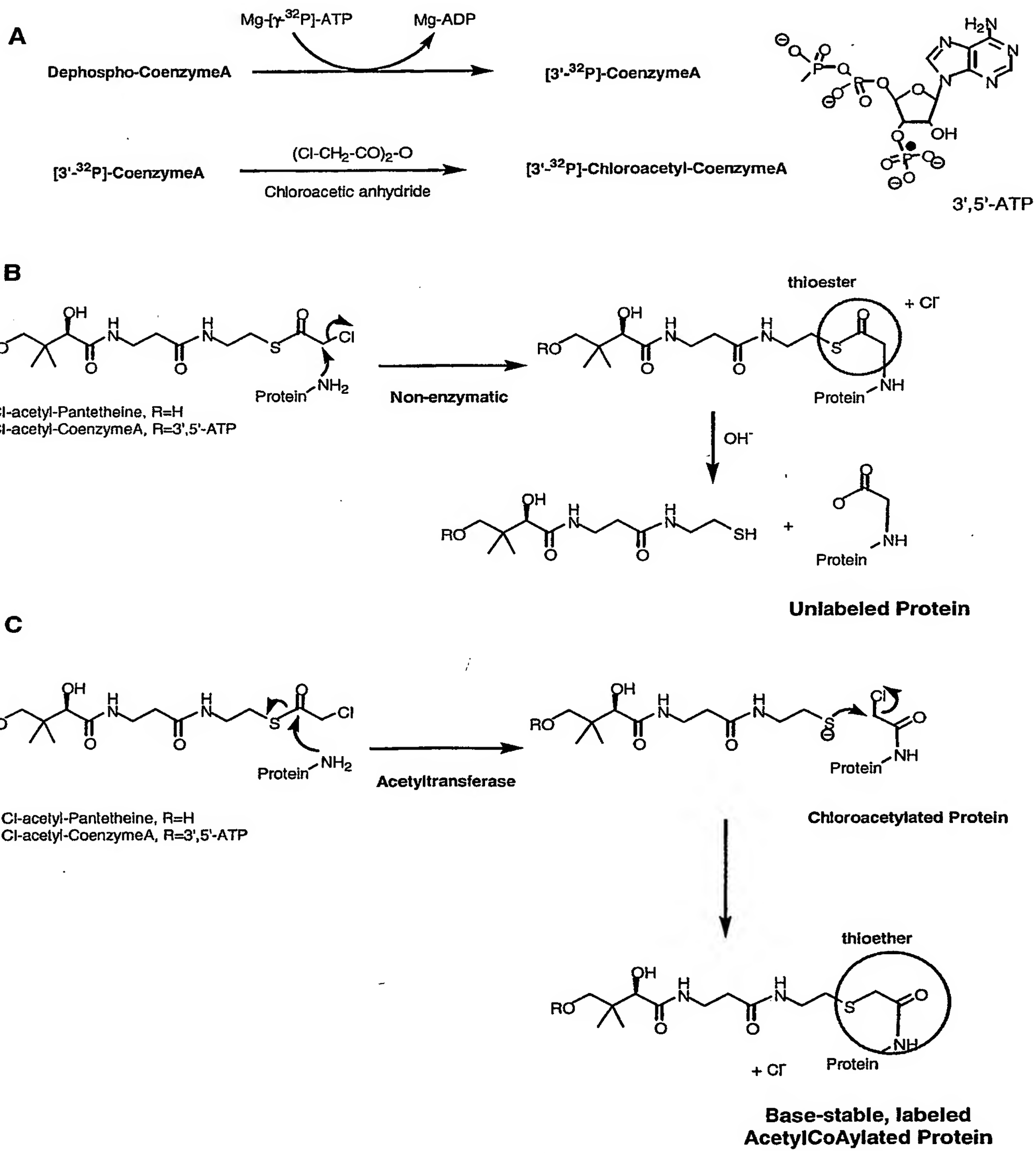

FIG. 2

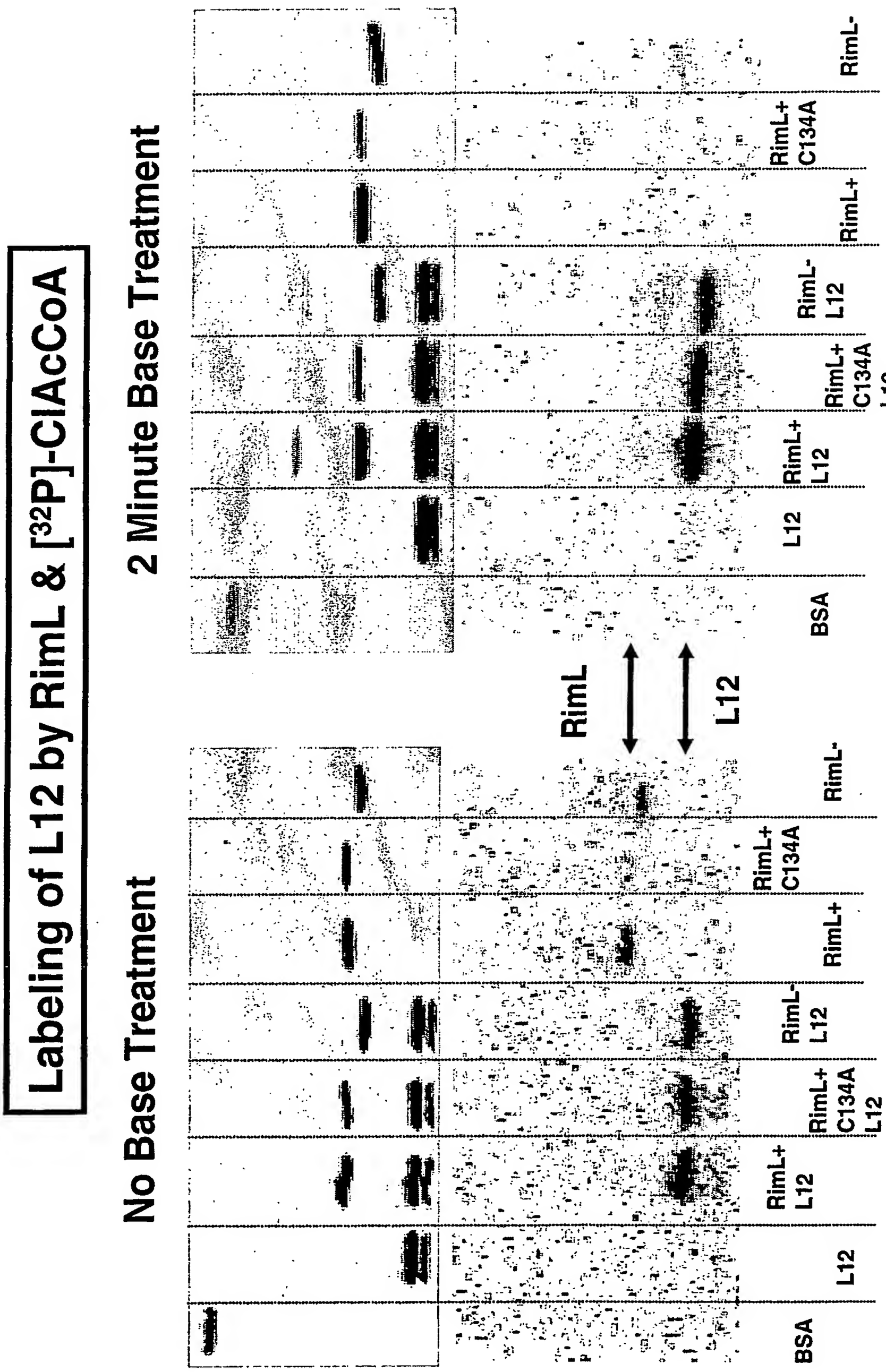
Labeling of L12 by RimL & [32P]-ClAcCoA
No Base Treatment
2 Minute Base Treatment
RimL
L12
BSA
L12
RimL+ L12
RimL+ C134A L12
RimL- L12
BSA
L12
RimL+ L12
RimL+ C134A L12
RimL- L12
Best Available Copy

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FIG. 3

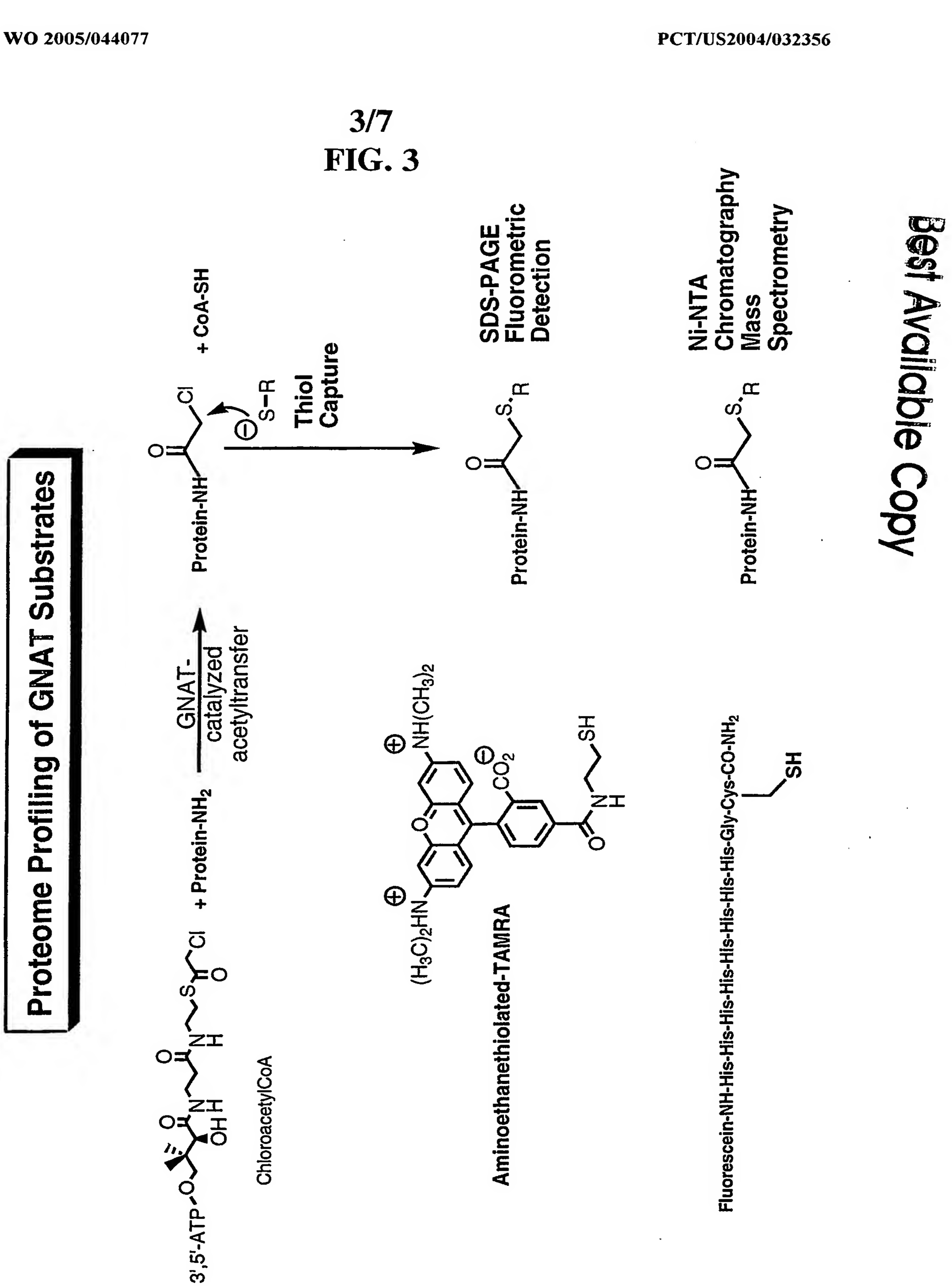
Proteome Profiling of GNAT Substrates
+ Protein-NH2
GNAT-catalyzed acetyltransfer
Protein-NH
+ CoA-SH
Thiol Capture
SDS-PAGE Fluorometric Detection
Ni-NTA Chromatography Mass Spectrometry
3',5'-ATP
ChloroacetylCoA
Aminoethanethiolated-TAMRA
Fluorescein-NH-His-His-His-His-His-His-Gly-Cys-CO-NH2
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FIG. 4

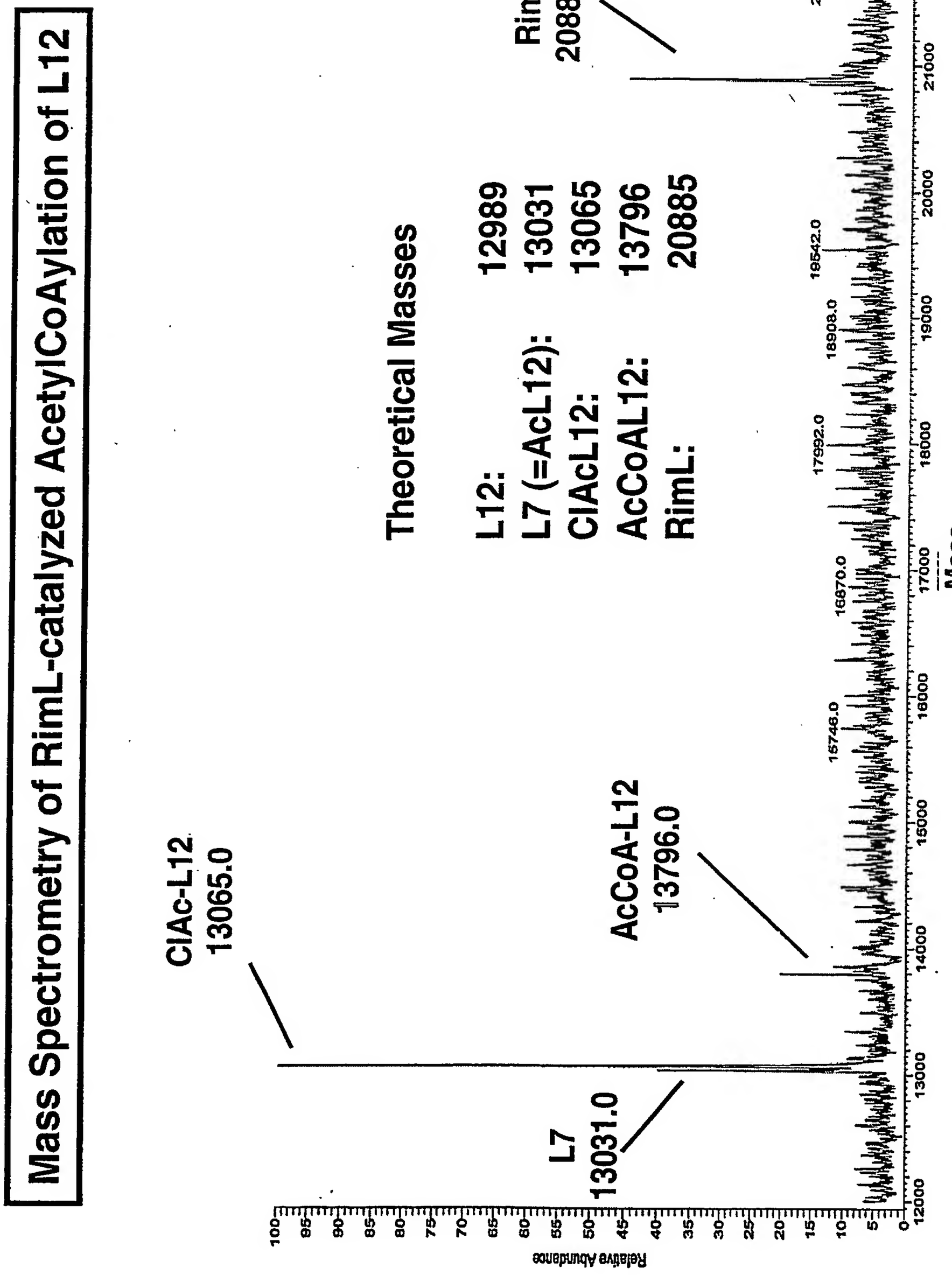
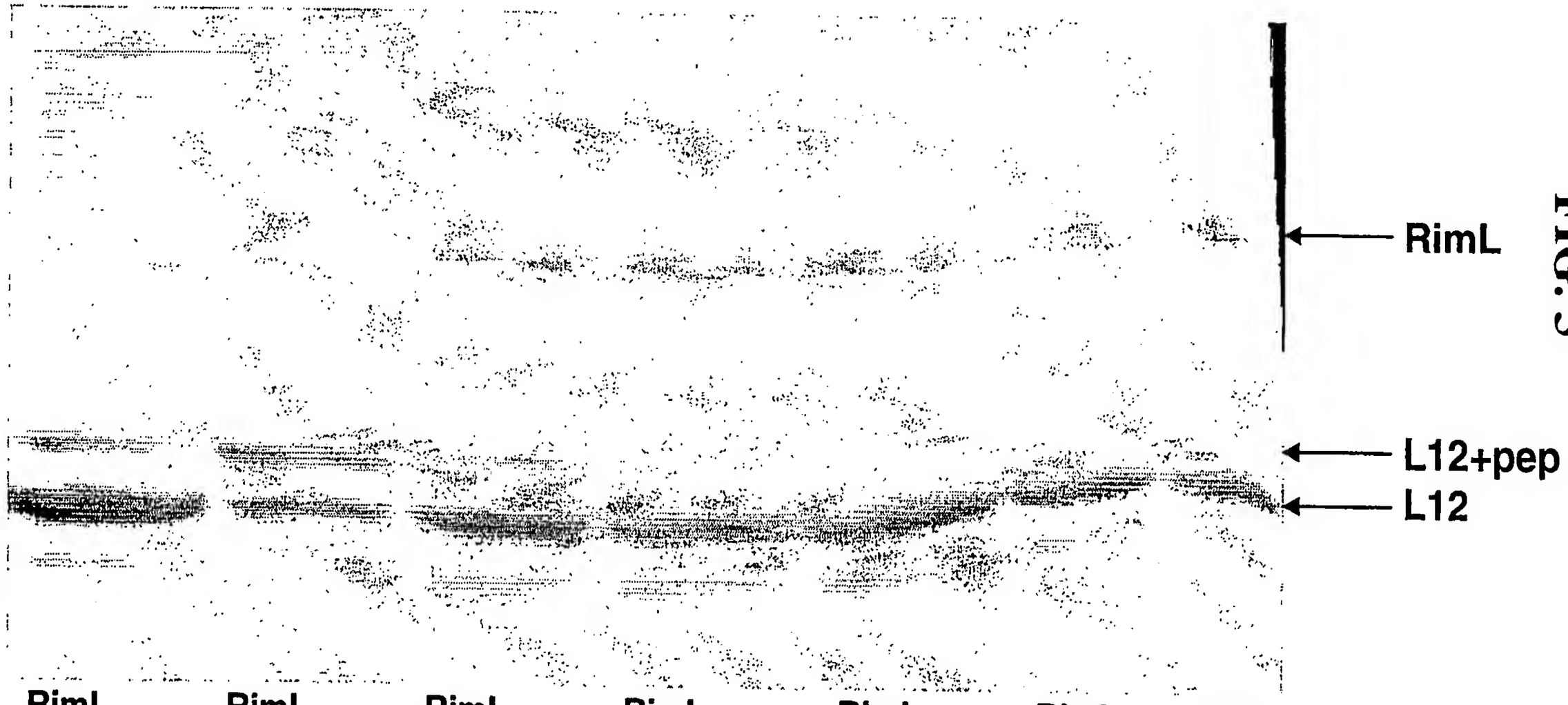

Mass Spectrometry of RimL-catalyzed AcetylCoA Acylation of L12
Theoretical Masses
L12: 12989
L7 (=AcL12): 13031
ClAcL12: 13065
AcCoAL12: 13796
RimL: 20885
ClAc-L12
13065.0
L7
13031.0
AcCoA-L12
13796.0
RimL
20885.0
Relative Abundance
Mass
Best Available Copy

FIG. 5

Affinity Labeling of Acetyltransferase Substrates

Cl-acetylation or Br-acetylation
50mM Tris, pH 7.5
RimL 2uM
L12 40 uM
ClAcCoA, BrAcCoA 250 uM
37°C for 1hr

Thiol capture peptide
100mM Tris, pH 8.5
1mM thiol peptide (HHHHHHGGC)
Different temperatures



RimL	RimL	RimL	RimL	RimL	RimL	RimL
L12	L12	L12	L12	L12	L12	L12
BrAcCoA	ClAcCoA	BrAcCA	ClAcCoA	BrAcCoA	ClAcCoA	
Peptide	Peptide	Peptide	Peptide			
5min	5min	22 °C	22 °C			
100 °C	100 °C	4hr	4hr			

FIG. 6

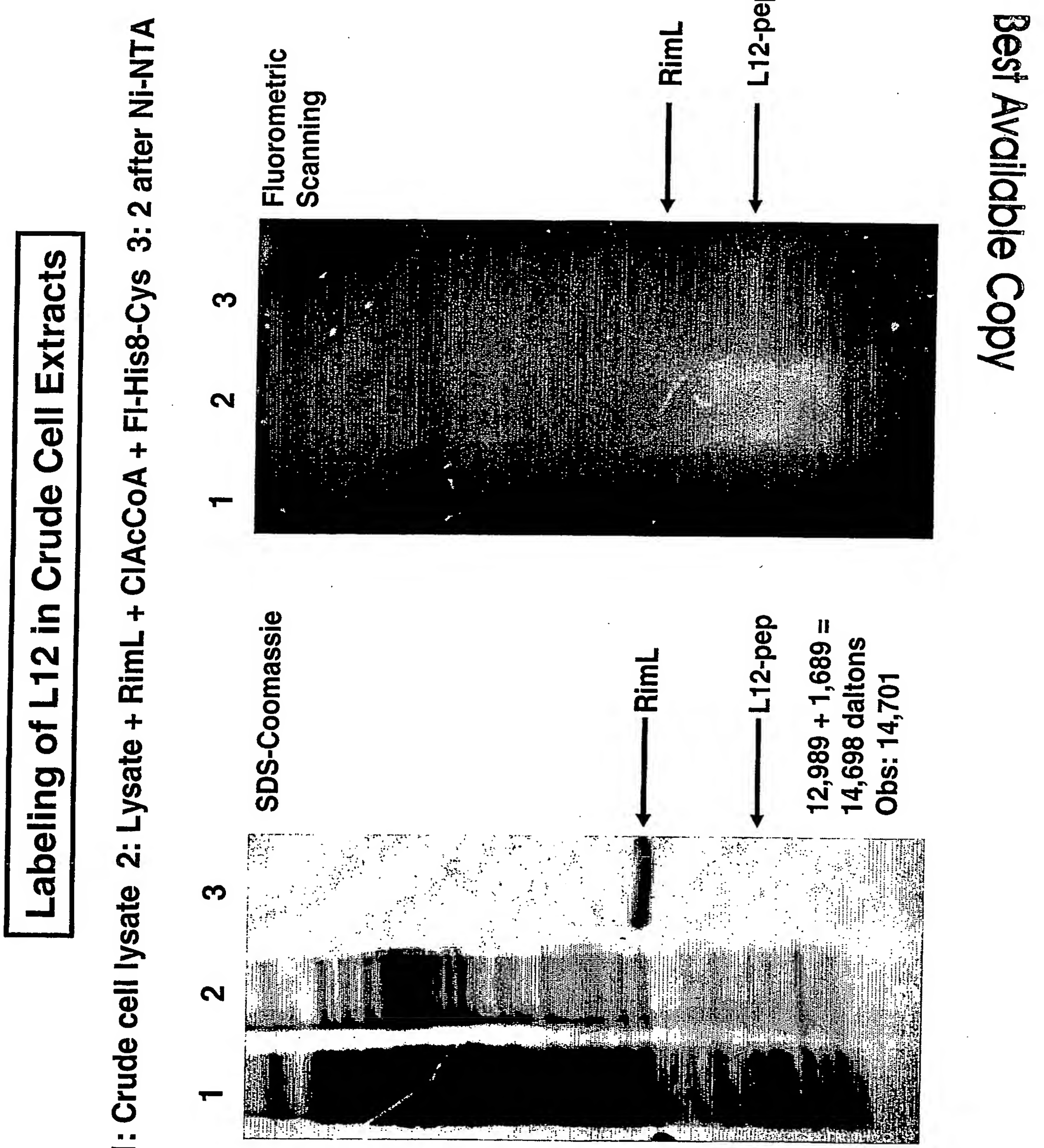

Labeling of L12 in Crude Cell Extracts
1: Crude cell lysate 2: Lysate + RimL + ClAcCoA + Fl-His8-Cys 3: 2 after Ni-NTA
SDS-Coomassie
RimL
L12-pep
12,989 + 1,689 = 14,698 daltons
Obs: 14,701
1 2 3
Fluorometric Scanning
RimL
L12-pep
1 2 3
Best Available Copy

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FIG. 7

Chloroacetylation of histones by Hat1 Acetyltransferase

Chloroacetylation

50 mM Tris pH 7.3
200 µM ClAcetylCoA
9 µM histones (0.8 mg/ml)
0.4 µM Hat1 (0.02mg/ml)

Thiol capture

3 mM TAMRA-CH_2CH_2SH
200 mM pH 8.4 Tris
4 hr